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GLUCOMANNANS OF *Serissa serissoides* STEM

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One β-*D-glucomannan was isolated from* Serissa serissoides *(DC.) Druce. The average molecular weight of the polysaccharide was established to be* 2.9×10^3 *Da. The structure of the polysaccharide from* S. serissoides *(SSP) was investigated by using sugar and methylation analysis, infrared spectroscopy, mass spectrometry, and NMR spectroscopy. The experiments revealed that SSP consisted of* β-*D-Glc*p *and* β-*D-Man*p *units in the ratio of approximately 1.1:1 with* β-*D-(1→4)-Glc*p *and* β-*D-(1→2) Man*p *linkages.*

Keywords: *Serissa serissoides* (DC.) Druce, polysaccharide, *β*-D-glucomannan, structural analysis.

Serissa serissoides (DC.) Druce belongs to the Rubiacea family and is widely distributed in China. It has been used as traditional medicine to remedy hepatitis and hepatocirrhosis in China for a long time. Bioactive investigation showed that the water extract of *S. serissoides* effectively inhibited secretion of HBsAg and HBeAg from the HepG 2.2.15 cell line [1] and protected mice liver from injuries from carbon tetrachloride, acetaminophen, and thioacetamide [2]. The water extract also increased the avoirdupois and thymus gland index of the tested mice [3] and showed antimicrobial activities [4]. Chemical investigations of *S. serissoides* led to the isolation of ursolic acid, 10-deacetyl asperulosidic acid, paedemsidic acid, oleanolic acid, palmitic acid, corosolic acid, urs-12-en-28-ol, 4-hydroxy-3-methoxybenzoic acid, 2,6-dimethoxy-*p*-benzoquinone, (–)-syringaresinol, (+)-medioresinol, vitexin, daucosterol, (+)-pinoresinol, (–)-syringaresinol-4-*O*-*β*-D-glucopyranoside, D-mannitol, *β*-sitosterol, and (–)-olivil from *S. serissoides* [5–7]. Investigation of polysaccharides from *S. serissoides* has not been reported. We described herein the isolation and structural analysis of the polysaccharide from the water extract of *S. serissoides*.

Polysaccharide SSP was isolated by water extraction. Crude polysaccharide was extracted by Sevag reagent to remove protein. Absence of a peak at 280 nm in the ultraviolet absorption spectrum revealed that the sample did not contain protein. Column gel permeation chromatography (Sephadex G-25) of the water-soluble sample yielded a pure *Serissa serissoides* polysaccharide (SSP). The total sugar content in SSP was estimated by the phenol–sulfuric acid method [8] and was found to be 99%. HPGPC showed only one symmetric peak, from which the weight-average molecular mass was estimated to be 2.9×10^3 Da by referencing to authoritative dextrans. Sugar analysis revealed the presence of Glc and Man by referencing to authoritative monosaccharides.

The results of GLC-MS analysis of partially methylated alditol acetates obtained from SSP identified three products: 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (*m/z*: 59, 87, 101, 113, 117, 131, 145, 161, 205), 1,2,5-tri-*O*-acetyl-3, 4,6-tri-*O*-methyl-D-mannitol (*m/z*: 43, 58, 87, 101, 129, 143, 161, 189, 233), and 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol (*m/z*: 59, 87, 99, 101, 113, 117, 113, 131, 157, 233) in a molar percentage of 19:38.3: 42.7 on the basis of peak areas. 1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol was derived from Glc*p* (1→) residue as a terminal in the polysaccharide. 1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol was derived from (1→4) Glc*p* residue, and 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl-D-mannitol from (1→2) Man*p* residues in SSP. SSP showed absorption bands at 890, 922, 1018, 1084, 1142, 2940, and 3420 cm^{-1} in the IR. The bands at 814, 873, and 897 cm^{-1} were ascribed to the *β*-type glycosidic linkage of Man*p* and Glc*p* [9]. The bands at 1018, 1084, and 1142 cm^{-1} were ascribed to the glycosidic linkage ν (C-O-C) and ν (C-O-H).

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TABLE 1. ^{13}C NMR Data of SSP (in D_2O)

Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6
→2)*β*-D-Man*p*(→1	103.8	74.5	81.1	77.5	77.2	62.3
→1)*β*-D-Gal*p*	103.3	72.6	74.5	77.0	77.0	60.7
→4)*β*-D-Glc*p*(1→	103.3	72.6	77.5	81.2	74.5	61.1

The ^{13}C NMR of SSP showed signals at 103.3–103.8 ppm (Table 1), confirming the *β*-type glycosidic linkage of anomeric carbon (C-1) in Man*p* and Glc*p* units [10, 11]. The signals at 81.2 and 81.0 ppm were respectively attributed to C-4 of the (1→4) Glc*p* unit and C-2 of the (1→2) Man*p* unit. The signal at 103.3 was attributed to C-1 in the (1→4) Glc*p* unit and in the (→1) Glc*p* unit, and the signal at 103.8 ppm was assigned to C-1 of the (1→2) Man*p* unit. The absence of signals in the range of 65–70 ppm showed that all the C-6 in the polysaccharide are not *O*-substituted [12, 13].

The structure of SSP was determined as *β*-D-Glc*p* (1→ [4]-*β*-D-Glc*p* (1→) →[→2]-*β*-D-Man*p* (1→).

EXPERIMENTAL

General. Ultraviolet spectra were recorded with a PC-2501 UV/VIS spectrometer (Shimadzu, Japan), and infrared spectra were obtained using a Nexus 470 infrared spectrometer (Thermo Nicolet, USA) with OMNIC 5.2 software; Gas chromatography–mass spectrometry was performed on a QP-55050A instrument (Shimadzu, Japan) equipped with fused silica capillary OV-225 (0.25 mm × 25 m) and a temperature program of 150°C to 230°C, and MS of the partially methylated alditol acetates was performed at 70 eV. GPC was performed with an Agilent HPLC apparatus (Agilent, USA) equipped with a TSK G-3000 SW column (300 × 7.5 mm), a model 410 refractive index detector, and a Millennium-32 Workstation. Dialysis was carried out in Spectra/Por 3 dialysis tubes (molecular weight cutoff 4000).

Materials. The plant was purchased from the medical herb market in Nanning, China. Sephadex G-25 (Pharmacia Biotech Limited, Denmark); D-arabinose, D-xylose, L-rhamnose, D-mannose, D-lyxose, D-glucose, D-galactose, D-fucose, and D-fructose (Sigma Chemical, USA); dextran P-112000, P-22800, P-11800, P-5900, P-2700 (Wako Chemical, Japan).

Isolation and Purification of Polysaccharide. Two kilograms of the plant material were extracted with 4000 mL ethanol (95%), then washed with distilled water and extracted with boiling water (4000 mL) for 10 hours. The extract was filtered and then concentrated to 1/8 volume under vacuum to give a residue (25.3 g). The residue was dissolved in 350 mL distilled water, extracted with 100 mL Sevag reagent ($CHCl_3$–*n*-BuOH 4:1, v/v) used repeatedly until no peaks were present at 280 nm in the ultraviolet absorption spectrum. The deproteinized residue was concentrated to 1/2 volume, and then precipitated in 95% ethanol under stirring. The precipitate was washed with ethanol repeatedly, then dissolved in distilled water and subsequently precipitated repeatedly. The precipitate was collected and lyophilized to give a water-soluble sample (8.5 g). The 500 mg sample was purified on a Sephadex G-25 gel column (2 × 50 cm) with 0.1 M solution of sodium chloride. The eluates were collected (2 mL per fraction) and monitored by the phenol-sulfuric acid method [14]. Fractions were combined and dialyzed to give purified *Serissa serissoides* polysaccharide (SSP, 460 mg).

Total Sugar Content. The total sugar content of SSP was determined by the modified phenol-sulfuric acid method using D-glucose as a reference [15].

Identification of Monosaccharide. SSP (10 mg) was hydrolyzed with 2 mol/L H_2SO_4 (3 mL) in a sealed tube at 100°C for 24 hours. The hydrolysate was neutralized with $BaCO_3$ and filtered successively. The filtrate and authoritative monosaccharides were subjected to chromatography on TLC plate with a solvent system EtOAc–AcHO–*n*-BuOH–H_2O (3:4:5:1, v/v), then visualized by spraying aniline/diphenylamine/phosphoric acid reagent [16].

Homogeneity and Molecular Weight. The homogeneity and molecular weight of SSP were determined by GPC with an Agilent HPLC apparatus (Agilent, USA) equipped with a TSK G-3000 SW column (300 × 7.5 mm), a model 410 refractive index detector, and a Millennium-32 Workstation [16] for the calculation of average molecular weights. The dextran standards (P-112000, P-22800, P-11800, P-5900, P-2700) were used for the calibration curve. The detailed experimental conditions are the following: column temperature 21°C (column temperature auto-control system); column pressure 5 Mpa (model 600 pump); injection volume 50.00 μL; sampling volume 20 μL; mobile phase AcOH–NaOAc buffer solution (pH 5); mobile rate 1.0 mL/min; and running time 45 min.

Methylation Analysis. 200 mg of SSP was methylated according to the method of Hakomori [17] to afford a product (28.5 mg) showing no absorption for hydroxyl at 3600–3300 cm^{-1} in its IR spectrum. The product was hydrolyzed with 90% formic acid for 6 hours at 100°C in sealed tubes, and then hydrolyzed with 2 mol/L trifluoroacetic acid for 6 h at 100°C in sealed tubes. The hydrolysate was then reduced with aqueous $NaBH_4$ to afford methylated alditol. The methylated alditol reacted with excess acetic anhydride to give methylated alditol acetate (12.4 mg). The methylated alditol acetates were analyzed by GC–MS with a fused silica capillary column under the temperature program. The temperature program started at 150°C with a 1 min hold, followed by a 3°C/min gradient to 200°C, then 5°C/min gradient to 270°C and finished with a 15 min hold at 270°C. The MS data were compared with data in the library to give the structure of methylated alditol acetate.

NMR Spectroscopy. SSP samples (30 mg) were dissolved in 0.5 mL D_2O. 125 MHz ^{13}C NMR spectrum was recorded on a Bruker AM500 (Germany) at 25°C, with TMS as external reference. The chemical shifts were recorded in ppm.

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